

Genetic and genomic diversity response of rubber tree to a major fungal disease

Le Guen V., Koop D.M., Salgado L.R., Déon M., Doaré F., Souza L.M., Seguin M., Berger A.,
Pujade-Renaud V., Garcia D.

Oral presentation as invited speaker at the 11th International Congress of Plant Molecular Biology,
Foz do Iguaçu, Brazil, 25th – 30th October 2015.

Introduction

Rubber tree is a perennial plant from the Euphorbiaceae family, and the genus *Hevea* which encompasses 10 species (Priyadarshan and Gonçalves 2003). All these species originate from the Amazon forest in the countries of Brazil, Bolivia, Peru, Colombia, Venezuela, French Guiana, Surinam and Guyana (Schultes 1990).

The cultivated species, *Hevea brasiliensis*, is far from a model species, with its very long life cycle, the area occupied by every single tree, and the big size of its genome: 2.0×10^9 nucleotides.

Rubber tree is cultivated for the production of latex which is harvested by incising the bark through a periodic agricultural practice called “tapping” (Webster and Baulkwill 1989). The main constituent of the latex is a long polymeric chain called cis-1,4-polyisoprene. Once collected in individual cups, the latex is coagulated, cleaned, dried and pressed to produce the raw material called “natural rubber” and used by several sectors of the industry.

During the last decades, worldwide natural rubber production has continuously progressed from around 2 million tons in 1961 to 12 million tons in 2013. On this total, 76% are produced by smallholders and 24% by large industrial estates. The tire industry is the main destination of this production, with 70% of its consumption.

Until the end of the 19th century, natural rubber was exclusively gathered on wild rubber trees in the Amazon forest. In 1876 thousands of seeds were collected by Henry Wickham in the state of Pará (Brazil) and sent to London where they were germinated in Kew Botanical Garden. Few germinated plantlets were afterwards sent to Colombo and Singapore where they were planted and grown until adult trees (Dean 1987). All subsequent rubber plantations in South East Asia originate from seeds of these first “Wickham trees”. The budding technique, which has been adapted to rubber tree and improved during the 1920s was used to select the elite trees in the existing plantations to produce the first generation of “Wickham clones”.

Though originated from South America, Asia accounts nowadays for about 92% of natural production, followed by Africa for 5% and South America for only 3%. The main explanation for this is the presence in South America of a lethal disease called South American Leaf Blight (SALB) caused by an Ascomycota fungus: *Microcyclus ulei*.

This disease remains until now endemic to the American continent. The fungus is an obligate biotroph which infects and develops on young leaves and thus may cause repeated defoliations on

susceptible cultivars (Chee and Holliday 1986). Trees are weakened by these defoliations and may die in few months. The chemical control is possible but not economically feasible nor desirable due to human health concern and environmental damages. The fungus *M. ulei* is characterized by a high diversity of physiological races. All Asiatic rubber cultivars (the so-called “Wickham clones”) are highly susceptible to SALB. However numerous genetic resistances can be found in *Hevea* spp. genetic pool, in natural wild populations or South American cultivars.

The life-cycle of this disease can be very fast (Chee and Holliday 1986). It starts with the infection of young leaves with conidia (asexual spores) or ascospores (sexual spores). This initial infection gives a sporulating lesion on the lower surface of the leaves that can in turn produce conidia within 11 days, giving start to an exponential development of the disease. After a more variable time lapse, the lesion can evolve and produce ascospores on the upper surface of the leaves.

SALB resistance and genetic structure of rubber tree populations

The genetic structure of wild rubber tree Amazonian populations from the Brazilian states of Acre, Rondônia and Mato Grosso has been investigated with microsatellite markers by Le Guen et al. (2009). A more recent study (Souza et al. 2015) included also populations from the Brazilian states of Amazonas and Pará (see Figure 1).

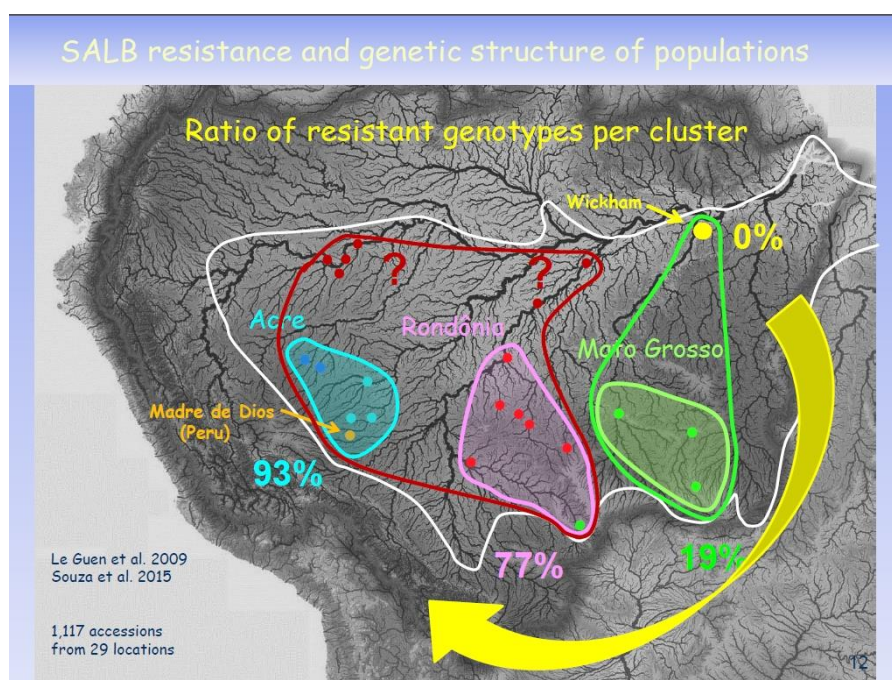


Fig 1 Ratio of resistant genotypes according to the different clusters of populations identified among wild rubber accession.

These two studies showed a partition of natural rubber Amazonian populations into two primary clusters, the first one composed of populations from Mato Grosso as well as Wickham clones, and the second one composed of all other populations from Rondônia, Acre, Amazonas, Madre de Dios, and Pará. Another study on field resistance to SALB (Le Guen et al. 2002) showed a gradient of SALB resistance from east to south-west of the area of origin of the species (19% of resistant accessions among Mato Grosso populations, 77% among Rondônia populations and 93% among Acre and Madre de Dios populations). This gradient of SALB resistance explains why among Wickham clones

originated from north-east of the area of origin it has never been encountered genotypes with SALB resistance.

Diversity of genetic factors among four resistant genotypes

This section focuses on four South-American rubber genotypes that exhibit various degrees of SALB resistance. These 4 genotypes and their main characteristics are described in the Table 1.

All these four genotypes have been crossed with a susceptible Wickham clone, to produce biparental populations segregating for this trait of SALB resistance. For each of the resulting segregating populations, a minimum of 150 individual progenies have been genotyped with numerous molecular markers and genetic linkage maps have been established. The same individual progenies have been observed for their response to SALB under controlled or natural conditions of infestation so that it was possible to identify major genes or quantitative trait loci (QTL) implied in the genetic resistance to SALB.

Genotype	Type of resistance to SALB	Geographical extension	References
RO38	Partial resistance from interspecific hybrid	Bypassed in Brazil and in French Guiana (2004)	(Lespinasse et al. 2000) (Le Guen et al. 2003)
FX2784	Complete	Bypassed in Bahia, durable elsewhere	(Le Guen et al. 2013)
MDF180	Partial resistance, no sexual form (teleomorph) of the fungus	Durable in Brazil and in French Guiana	(Le Guen et al. 2011)
FDR5597	Partial, from Madre de Dios origin	Durable in Brazil and in French Guiana	Not published

Table 1. Main characteristics of the SALB resistant parents of the four mapping populations

These major genes and QTLs are shown in Figure 2

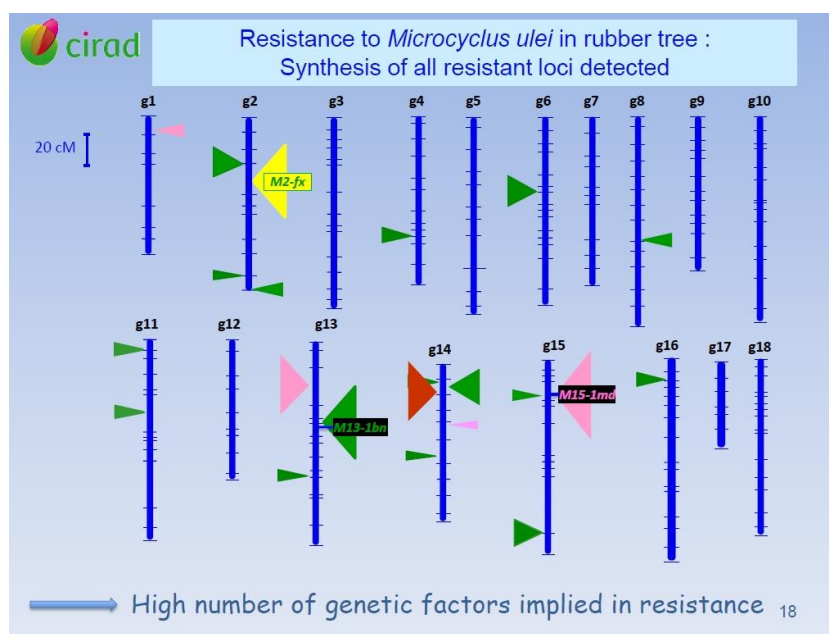


Fig 2. Localization of major genes and QTLs for SALB resistance in the genome of four genotypes: RO38 (green), MDF180 (pink), FX2784 (yellow) and FDR5597 (brown).

The synthesis of these results illustrates the great complexity of the different forms of SALB resistance, as well as for some of them an unexpected genetic determinism. It could be noticed that either complete or partial, specific or general, SALB resistance could be monogenic or multi-genic. Furthermore, we also observed that durable resistance could be governed by few loci, and also that a multi-genic resistance could be bypassed by *M. ulei*.

Diversity of genes implied in resistance to SALB.

Aiming at identifying the global diversity of genes that are implied in SALB resistance, different gene expression analyses were performed.

First of all, a qualitative gene expression analysis by EST profiling was carried out by assessing differentially expressed genes in Suppression Subtractive Hybridization (SSH) libraries (Garcia et al. 2011). For two genotypes (resistant versus susceptible) the transcripts produced on tester plants (on which leaves were inoculated by the fungus) were subtracted by the transcripts produced on driver plants (non-inoculated leaves). Up regulated transcripts were isolated at 3 different post-infection stages: early (6-72 h post infection), late (4 to 28 days p.i.) and very late (1 to 2 months p.i.). About 1600 expressed sequence tags were produced through this procedure on which the following observations could be made:

- Few common transcripts could be observed between resistant and susceptible libraries
- The genes triggered by infection were different according to stage p.i.
- Genes of defense against stress, as well as genes associated with oxidative stress were up regulated
- The overall antioxidant activity was more complete and up-regulated in resistant genotypes

A second study aimed at quantifying by real-time PCR the activity of some genes formerly identified (Koop et al. submitted). Two resistant genotypes differing for their resistance type (the above mentioned FX2784 and MDF180 cultivars) and one susceptible genotype were inoculated with *M. ulei* strains. The infected leaves were sampled at 5 post-infection stages, their RNA were extracted, cDNA were synthesized and rt-PCR was carried out using primers specific from two gene families. The two studied families were:

- Ethylene synthesis and signaling pathway (ERF)
- Reactive Oxygen Species (ROS) scavenging enzymes

In the ERF family, 31 genes could be quantified by rt-PCR on the 3 genotypes (see Figure 3).

For the totally resistant genotype FX2784, we could observe an over-expression of numerous ERF genes 2 days after infection. These genes may have a function in activating pathogen defense genes.

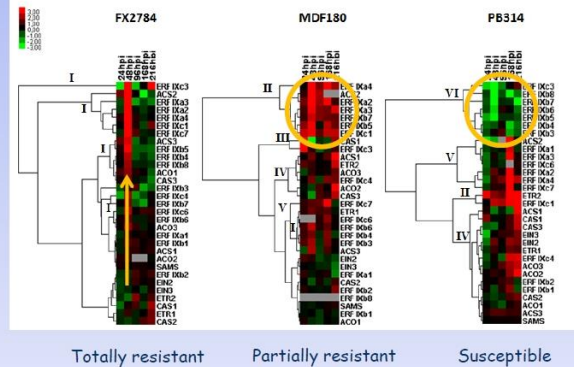
For partially resistant MDF180, a cluster of 7 ERF genes were durably over-expressed from 2 days to 9 days after infection.

Gene expression analysis (Koop et al. submitted)

Ethylene synthesis and signaling pathway (ERF)

31 genes quantified by rtPCR

Figure 3: Hierarchical clustering illustrating genes related to ethylene synthesis and ethylene signaling pathways, co-ordinately expressed in three *Hevea* genotypes in response to *M. ulmi* infection. For each gene, the fold change of the mRNA level is represented by red or green, indicating up-regulation or down-regulation, respectively. The differentially expressed genes were classified into six regulatory patterns (clusters), indicated by numbers I through VI.



22

Fig 3 Hierarchical clustering representation of expressed genes related to ethylene synthesis and ethylene signaling pathway.

For susceptible PB314, other 7 ERF genes were down regulated from 48 hours to 9 days after infection, and other ERF and CAS genes are up-regulated from 7 to 9 days after infection. This activation occurred too late and for this reason was inefficient for controlling the spread of mycelia of the fungus inside the leaf tissues.

In the Figure 4 below were presented the results of quantification of the expression of genes related to ROS production and scavenging. The important figure is the high and constant expression of HbWRKY2 in partially resistant genotype induced at early stage. Worky transmission factors were described in model plants as key regulators in the response to biotic and abiotic stresses.

Conclusion

The next steps towards identifying genes implied in resistance to SALB will certainly be to quantify more in depth the 81 genes of the Worky family that have been recently described in rubber tree by Li et al. (2014), and also the 114 ERF genes identified by Piyatrakul et al. (2014). It should be also interesting to investigate the polymorphism within transcription factor candidate genes, to investigate the co-localization of resistance QTLs and these TF candidate genes, and then to incorporate these newly identified genes into breeding schemes for Molecular Assisted Selection.

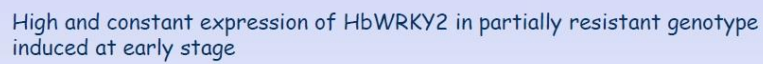


Fig 4 Hierarchical clustering representation of genes related to ROS production and scavenging in 2 resistant and 1 susceptible rubber tree genotypes

References

Chee KH, Holliday P (1986) South American leaf blight of *Hevea* rubber, MRRDB edn, Kuala Lumpur

Dean W (1987) Brazil and the struggle for rubber. Cambridge University Press, Cambridge UK

Garcia D, Carels N, Martins Koop D, Araujo de Souza L, De Andrade Junior SJ, Pujade Renaud V, Mattos CRR, Cascardo JCM (2011) EST profiling of resistant and susceptible *Hevea* infected by *Microcyclus ulei*. *Physiological and Molecular Plant Pathology* 76:126-136

Le Guen V, Doaré F, Weber C, Seguin M (2009) Genetic structure of Amazonian populations of *Hevea brasiliensis* is shaped by hydrographical network and isolation by distance. *Tree Genetics and Genomes* 5:673-683

Le Guen V, Garcia D, Doaré F, Mattos CRR, Condina V, Couturier C, Chambon A, Weber C, Espéout S, Seguin M (2011) A rubber tree's durable resistance to *Microcyclus ulei* is conferred by a qualitative gene and a major quantitative resistance factor. *Tree Genetics and Genomes* 7:877-889

Le Guen V, Garcia D, Mattos C, Fouet O, Doaré F, Condina V, Seguin M (2013) A newly identified locus controls complete resistance to *Microcyclus ulei* in the Fx2784 rubber clone. *Tree Genetics & Genomes* 9:805-812

Le Guen V, Garcia D, Mattos CRR, Clément-Demange A (2002) Evaluation of field resistance to *Microcyclus ulei* of a collection of Amazonian rubber tree (*Hevea brasiliensis*) germplasm. *Crop Breeding and Applied Biotechnology* 2:141-148

Le Guen V, Lespinasse D, Oliver G, Rodier-Goud M, Pinard F, Seguin M (2003) Molecular mapping of genes conferring field resistance to South American Leaf Blight (*Microcyclus ulei*) in rubber tree. *Theoretical and Applied Genetics* 108:160-167

Lespinasse D, Grivet L, Troispoux V, Rodier-Goud M, Pinard F, Seguin M (2000) Identification of QTLs involved in the resistance to South American leaf blight (*Microcyclus ulei*) in the rubber tree. *Theoretical and Applied Genetics* 100:975-984

Li H-L, Guo D, Yang Z-P, Tang X, Peng S-Q (2014) Genome-wide identification and characterization of WRKY gene family in *Hevea brasiliensis*. *Genomics* 104:14-23

Piyatrakul P, Yang M, Putranto R-A, Pirrello J, Dessailly F, Hu S, Summo M, Theeravatanasuk K, Leclercq J, Kuswanhadi, Montoro P (2014) Sequence and Expression Analyses of Ethylene Response Factors Highly Expressed in Latex Cells from *Hevea brasiliensis*. *PLoS ONE* 9:e99367

Priyadarshan PM, Gonçalves PS (2003) *Hevea* gene pool for breeding. *Genetic Resources and Crop Evolution* 50:101-114

Schultes RE (1990) A brief taxonomic view of the genus *Hevea*, MRRDB edn, Kuala Lumpur

Souza LM, Le Guen V, Cerqueira-Silva CBM, Silva CC, Mantello CC, Conson ARO, Vianna JPG, Zucchi MI, Scaloppi Junior EJ, Fialho JdF, de Moraes MLT, Gonçalves PdS, Souza APd (2015) Genetic Diversity Strategy for the Management and Use of Rubber Genetic Resources: More than 1,000 Wild and Cultivated Accessions in a 100-Genotype Core Collection. *PLoS ONE* 10:e0134607

Webster CC, Baulkwill WJ (1989) Rubber, Longman edn. Longman Singapore Publishers Ltd